Journal Pre-proof

Messenger Ribonucleic Acid Vaccines for Severe Acute Respiratory Syndrome Coronavirus-2 – A Review

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Abbreviations

Abbreviation	Definition	Abbreviation	Definition
COVID-19	Coronavirus disease of 2019	B.1.1.7	Alpha variant
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2	B.1.351	Beta variant
mRNA	Messenger ribonucleic acid	COVID-NET	COVID-19-Associated Hospitalization Surveillance Network
LNP	Liposomal nanoparticle	VOC	Variants of concern
SAM	Self-amplifying mRNA	UTR	Untranslated regions
dsRNA	Double-stranded RNA	PAMPs	Pathogen-associated molecular patterns
Ad5	Adenovirus type 5	MHC	Major histocompatibility complex
APCs	Antigen presenting cells	DCs	Dendritic cells
RBD	Receptor-binding domain	ACE-2	Angiotensin converting enzyme receptor
MERS-CoV	Middle east respiratory syndrome coronavirus	CVnCOV	CureVac

GMT	Geometric mean titer	GMC	Geometric mean concentration
BMI	Body mass index	FDA	Food and Drug Administration
EUA	Emergency use authorization	VAERS	Vaccine adverse event reporting system
CDC	Centers for Disease Control	BAU	binding antibody units

I. Background

Conventional vaccines, including inactivated, live attenuated, subunit, and conjugated and unconjugated polysaccharides, have long demonstrated great efficacy at preventing many infectious diseases and have made substantial global impacts to reduce morbidity and mortality due to preventable diseases. With the advent of Coronavirus disease of 2019 (COVID-19) pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), there was an urgent need for rapid, large-scale development and deployment of vaccines against this virus to curb the pandemic. However, production of conventional vaccines can be a time-consuming process that is often hampered by dependence on the production of cell lines and constructing protein subunits with high purity and reproducibility, which can often take more than one year.[1] Messenger ribonucleic acid (mRNA) vaccines can be manufactured rapidly at low cost due to high yields of *in vitro* transcription reactions and have additional benefits over traditional available vaccines.[2,3] For example: mRNA vaccines do not rely on animal products or cell cultures and because mRNA is a non-infectious platform that does not enter the nucleus or incorporate into the genome, thereby avoiding genomic insertion, it provides attractive safety characteristics.[1,4] For these reasons, mRNA vaccines were considered as one of the frontrunners for vaccine development against SARS-CoV-2 infection, especially in the setting of a pandemic.

The concept of utilizing exogenous mRNA to cause cells to produce proteins of interest in organisms was first described in the early 1970s.[5,6] *In vitro* studies involving injection of encephalomyocarditis virus RNA and mRNA encoding rabbit hemoglobin into oocytes from *Xenopus laevis* demonstrated that introduction of RNA molecules into cells could result in protein production.[5,6] The development of mRNA therapeutics made major strides in 1989 when researchers at the Salk Institute at University of California San Diego first described successful transfection of mRNA in a liposomal nanoparticle (LNP) into the cytoplasm of mouse cells.[7] A few months later, Wolff et al demonstrated that *in vivo* transfection of naked mRNA into mouse skeletal muscle resulted in detectable production of the encoded protein.[8] By 1993, Martinon et al showed that virus-specific cytotoxic T lymphocytes could be elicited *in vivo* by immunizing mice with

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liposome-entrapped mRNA encoding the nucleoprotein of the influenza virus.[9] By 2017, the first proof-of-concept phase 1 human trial of an mRNA vaccine against an infectious disease, the rabies virus, demonstrated the ability of this platform to induce an immune response to the encoded antigen of interest.[10] These early successes supported mRNA technology as a highly versatile and viable contender in ongoing efforts toward innovative vaccine development.

II. Expression System/Mechanism

mRNA is a single stranded RNA, and an intermediate transporter of the genetic code, that plays a key role between translation of protein-encoding DNA to protein production by ribosomes in the cytoplasm.[2,11] Functional synthetic mRNA is the result of *in vitro* transcription of a linearized plasma DNA template by a DNA-dependent RNA polymerase, such as the T7, T3 or Sp6 bacteriophage RNA polymerase, in the presence of nucleoside triphosphates.[11–14] The product is a functional synthetic mRNA that resembles mature mRNA found in eukaryotic cells, and the template DNA is subsequently degraded by DNases.[2,4,11] There are two types of mRNA utilized in the current vaccines under development: non-replicating mRNA and self-amplifying mRNA (SAM). Conventional non-replicating mRNA contains a 5' cap, an open reading frame containing the target protein sequence, 5' and 3' untranslated regions (UTRs), and a poly(A) tail.[2,4] SAM contains these essential elements, but it also encodes viral replication machinery which enables intracellular RNA amplification thereby increasing protein expression compared with non-replicating RNA.[2,15]

The process of *in vitro* transcription results in many byproducts, including contaminating double-stranded RNA (dsRNA), that can provoke an undesirable innate immune response and hasten the degradation of the newly made RNA.[11] By acting as pathogen-associated molecular patterns (PAMPs), these byproducts are detected by the immune system and can incite production of type I interferon which can lead to degradation of both the cellular and ribosomal RNA.[2,16] Additionally, the intrinsically immunogenic nature of RNA itself can provoke such an immune response and thus the process of purification of the mRNA becomes an important step.[3] Purification also plays a role in optimizing the of expression of mRNA once it enters the cell.[3] Several methods of removing the contaminating dsRNA have been described, including fast protein liquid chromatography, high-performance liquid chromatography, and nucleoside modification.[17] Utilization of these techniques facilitates maximal production of the desired protein and avoidance of adverse activation of innate immune responses.

The post-transcription process provides the essential modifications that impart structural stability and enhanced expression of the mRNA code. The 5' cap and poly(A) tail are essential for efficient protein translation and stabilization of the mRNA molecule in the cytoplasm.[11,18,19] The 5' and 3' UTRs have important structural roles as they provide the mRNA molecule with stability and regulate translation.[2,18] The 5' and 3' UTRs also prolong the half-life and enhance expression of the mRNA.[20] The post-transcription cytoplasmic mRNA then encounters the cell's translation machinery and the protein of interest is produced.[11] This protein then undergoes additional post-translational modifications, including proper folding, to make a fully functional protein.[11]

Other methods of increasing protein translation and expression have been described.[2] Sequence optimization and modification of codon usage by substituting rare codons for synonymous codons can increase transfer ribonucleic acid (tRNA) in the cytoplasm.[2,4,21] Another method described involves increasing the guanine and cytosine sequence content of the genetic code, which leads to increased steady-state mRNA levels resulting in increased gene expression.[2,15,22]

III. Delivery:

Once delivered at the site of injection, the mRNA in the vaccine must avoid degradation by nucleases and cross the cell's plasma membrane to reach the translation machinery in the cytoplasm in order to effect an antigen-specific immune response.[15,23] Figure 1 summarizes the mechanism of non-replicating mRNA vaccines against COVID-19. Multiple studies have demonstrated the ability of naked mRNA to be taken up by cells, however carrier systems to protect the mRNA from degradation enhance delivery from the site of injection to the cytoplasm.[11] There is also evidence that suggests both humoral and cell-mediated immune responses are improved with intramuscular delivery of LNP-formulated mRNA compared with naked mRNA.[3] Several carrier systems have been designed to optimize this process, among which LNP formulations are currently the most promising.[2,24] Given the intrinsically negative charge of the cell membrane, due to its phospholipid bilayer composed of polar heads and hydrophobic tails, the negatively charged mRNA benefits from the shelter provided by the positively charged LNP delivery systems to efficiently enter the cell.[15] LNPs are generally composed of an ionizable cationic lipid, which facilitates endosomal escape of the mRNA once in the cytoplasm, polyethylene glycol and zwitterionic lipids, which provide structural support and increase the half-life of the formula, and cholesterol, a stabilizing agent.[2,24,25] Initially this delivery system was studied for small interfering RNA

molecules, however it has subsequently been shown to be a successful and well tolerated method for both non-replicating mRNA and SAM delivery.[26,27]

Several studies have evaluated different routes of delivery of mRNA vaccines including intravenous, intradermal and intramuscular.[3] When LNP-mRNA is delivered intravenously it binds with high affinity to hepatocytes in the liver, whereas intradermal and intramuscular delivery resulted in longer expression of the antigen at the site of inoculation, with intradermal delivery resulting in the longest lasting expression.[3,28,29] After intramuscular injection of LNP-mRNA in mice and non-human primates, the mRNA is taken up and strongly expressed by local cells including professional antigen presenting cells (APCs), monocytes, granulocytes and dendritic cells (DCs), which may facilitate transport of the antigen to neighboring draining lymph nodes.[29] The professional APCs and DCs play a key role in processing the mRNA encoded antigen, presenting it on major histocompatibility complex (MHC) class I and II molecules to CD4 and CD8 T-cells, and facilitating recognition by B cells for the development of a humoral immune response.[3]

IV. Preclinical Data:

In preclinical studies, mRNA vaccines demonstrated a good safety profile, remarkable efficacy, long-lived immune responses to infectious pathogens and adaptability for emerging infectious diseases.[2] Multiple studies in animal models demonstrated that mRNA vaccines were able to elicit strong immunity against a variety of infectious diseases, including Zika virus, influenza, *Toxoplasma gondii*, Ebola, Powassan virus and rabies.[2,30–32] mRNA vaccines demonstrated their ability to provoke a robust immune response in the form of both neutralizing antibodies as well as CD8+ and CD4+ T cell responses. A study of an mRNA vaccine encoding the full-length hemagglutinin of influenza A (PR8HA) demonstrated not only the production of protective humoral immunity but also B and T-cell mediated immune responses in both young and old mice.[33] Few direct comparisons of immunogenicity and durability of protection have been made between mRNA vaccines and other vaccine constructs to date. There is some evidence that mRNA vaccines are more immunogenic with comparable durability of response as other vaccine constructs in preclinical studies. A single dose of the nucleoside-modified pre-membrane and envelope (prM-E) LNP-formulated mRNA vaccine construct against Zika virus (ZIKV prM-E mRNA-LNP) provoked higher neutralizing antibody responses than those elicited by plasmid DNA vaccines in mice and rhesus macaques.[34,35] Compared with a single 1 mg dose of DNA-vaccine, a single 50 μg dose of ZIKV prM-E mRNA-LNP induced RVP neutralizing antibodies (Nab) that were 50 times higher, and over twice as

high as 2 doses of DNA vaccine in macaques.[36] It also demonstrated that plaque reduction neutralization test ($PRNT_{50}$) NAb continued to increase over several months and were 50-100 times higher than those provoked by a single dose of purified inactivated virus or plasmid DNA vaccine.[34] Neutralizing antibody titers were stable through 12 weeks post-immunization.[34] Comparative evaluations of vaccine-induced NAb for ZIKV prM-E mRNA-LNP, an adenovirus vectored vaccine (RhAd52 ZIKV) and PIV in macaques was difficult due to use of different assays.[34,35] Another study demonstrated a stronger $CD4^+$ T cell response after RABV-G mRNA vaccine compared with the inactivated rabies vaccine (Rabipur) in mice.[31] In newborn piglets, the RABV-G mRNA vaccine lead to prompt seroconversion and comparable Nab titers as the Rabipur vaccine.[31] Non-human primates who received an LNP-complexed mRNA vaccine against influenza were demonstrated to have equivalent antibodies following one dose as compared with an adjuvanted subunit influenza vaccine, higher antibody responses after the second LNP-mRNA dose.[29] In rhesus macaques, vaccination with LNP-mRNA compared to adjuvanted HIV-1 Env recombinant protein vaccination showed similar or higher levels of HIV-1 Env-specific antibodies.[37]

Since the half-life and durability of non-replicating mRNA is transient, SAM vaccines aim to increase the expression of the encoded protein via their autoreplicative capabilities.[38(p7)] Comparisons between SAM and non-replicating mRNA vaccine efficacy are few. Vogel et al showed that equivalent levels of protection against influenza A and antigen expression were achieved after just 1.25 μg of SAM compared with 80 μg of non-replicating mRNA (a 64-fold difference in dose), thereby supporting the potency of SAM vaccine constructs.[39]

Prior studies of coronaviruses demonstrated that the neutralizing antibodies to the spike protein (S protein), which facilitates viral attachment and entry into the host cells, as well as passive transfer of S protein antibodies, provided protection against infection with this family of viruses in challenge models.[40,41] In SARS-CoV-2, the trimeric spike protein utilizes part of the N-terminal furin cleavage fragment (S1) called the receptor-binding domain (RBD) to bind to the angiotensin converting enzyme 2 (ACE-2) receptor on target cells.[42,43] Using the fusion machinery of the C-terminal furin cleavage fragment (S2), the S protein undergoes significant conformational changes in order to fuse the virus with the host cell membrane and allow entry of the viral genome, resulting in pre-fusion and post-fusion states of the protein.[1,42,43] Prior studies with a stabilized respiratory syncytial virus (RSV) subunit vaccine candidate demonstrated that utilizing the pre-fusion conformation of the RSV glycoprotein resulted in a significant increase in neutralizing antibody production to key epitopes, and demonstrated that methods to stabilize the S-protein in the pre-fusion state increases S-protein expression.[44,45] In studies on middle east respiratory syndrome coronavirus (MERS-CoV), SARS-CoV, and human coronavirus HKU1 (CoV-HKU1), it was discovered that 2 proline substitutions (K986P and V987P) in the apex of the central helix of the spike protein prevented post-fusion structural changes and effectively stabilized the S proteins in the pre-fusion conformation, termed the 2-SP (or 2P) design.[45] In a study involving MERS-CoV in a mouse model, immunization with MERS-CoV-S(2P) mRNA-LNP was protective against lethal MERS-

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CoV challenge in a dose-dependent manner.[46] To date, three mRNA vaccines against SARS-CoV-2 utilize the S-2P design, the mRNA-1273 (Moderna/NIH), BNT162b2 (Pfizer-BioNTech), and CVnCOV (CureVac).

On January 10, 2020 the genomic sequence of SARS-CoV-2 was released and a race to produce a vaccine based on the pathogen sequence began in order to gain a foothold on the rapidly rising global cases of COVID-19.[1] Figure 2 shows a timeline summarizing key events in COVID-19 mRNA vaccine development. Within 24 hours of the release of the SARS-CoV-2 genomic sequence, the S-2P design was utilized to produce the stabilized pre-fusion SARS-CoV-2 S(2P) protein *in silico* for analysis of the viral structure and development of viral assays.[1,47–49] Of all the available platforms of vaccine, the manufacturing advantages of mRNA vaccines allowed them to enter promptly into preclinical studies and two vaccine candidates, the BNT162b2 and mRNA-1273, were the front-runner mRNA based COVID-19 vaccine candidates. Other front runner vaccine candidates included Ad26.COV2-S (Janssen Pharmaceuticals/Johnson & Johnson), ChAdOx1 nCoV-19 (Oxford/AstraZeneca), and CoronaVac (Sinovac Biotech). A summary of the preclinical data from the Pfizer-BioNTech and Moderna COVID-19 vaccine products can be found on Table 1 and is discussed below.

Pfizer-BioNTech:

The vaccine development program by Pfizer-BioNTech included 4 investigational products, which differed in the nucleotide sequences of the encoded antigens, as part of their mRNA-based vaccine program, named Project Light Speed, in response to the COVID-19 pandemic.[50] The initial objective was to select a single vaccine candidate and dosing regimen to progress into further studies on safety and efficacy.[50]

The preclinical studies of BNT162b2 in mice and rhesus macaques demonstrated development of potent immunogenicity after vaccination. The BNT162b2 is an LNP-complexed N^1-methyl-pseudouridine (m1Ψ) nucleoside-modified mRNA encoding the full-length pre-fusion stabilized SARS-CoV-2 S-2P.[51] These modifications, along with non-coding sequence optimization, were shown to increase RNA translation *in vivo* with the added benefit of decreasing immune sensing.[28,51,52] Studies in BALB/c mice showed that strong neutralizing antibody production to S1 and the receptor binding domain (RBD) developed in a dose-dependent fashion after administration of 0.2 µg, 1 µg or 5 µg of BNT162b2, as well as systemic T-cell responses.[51] In mice, the prime-only regimen also demonstrated a strong neutralizing antibody and T cell responses.[51]Additionally, studies in rhesus macaques showed that a prime/boost vaccination of 100 µg of BNT162b2 resulted in SARS-CoV-2 neutralizing geometric mean titers (GMT) 18.0 times that seen in convalescent human serum when measured 7 days post dose 2.[51] Prime/boost vaccination of rhesus macaques with 100 µg of BNT162b2 successfully prevented lung infection after re-

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challenge of SARS-CoV-2 at a high intranasal and intratracheal inoculum (1.05 x 10^{6} PFU of SARS-CoV-2 USA-WA1/2020 isolate).[51] Prime/boost with 30 µg or 100 µg also demonstrated robust neutralizing antibody production as well as CD4 and CD8 T cell responses.[51]

Studies on another vaccine investigational project as part of Project Light Speed, BNT162b1, also showed promising results. It was composed of the same LNP-formulated m1Ψ nucleoside-modified RNA platform as BNT162b2 but expressed a T4 fibritin-derived trimerised version of the RBD to increase immunogenicity.[53] Similarly to BNT162b2, BNT162b1 elicited potent humoral and T-cell mediated immune responses in both mice and macaques in preclinical studies, and protected macaques against rechallenge with SARS-CoV-2.[51] Both the BNT162b1 and BNT162b2 moved forward into phase I clinical trials.

Moderna:

The mRNA-1273, a mRNA based vaccine encoding the full-length pre-fusion stabilized SARS-CoV-2 S-2P encapsulated in a LNP (containing 4 lipids including a novel proprietary lipid SM-102), demonstrated protective immune responses and good efficacy in preclinical trials.[1] The ability of S-2P antigen to be delivered by mRNA was confirmed *in vitro* in mouse models.[1] Assessments of immunogenicity in BALB/c mice after a prime/boost regimen of mRNA-1273, 0.01 µg or 0.1 µg or 1 µg dose, separated by 3 weeks, showed that S-binding and neutralizing antibodies were induced by the vaccine in a dose-dependent manner.[1] A wide dose range utilizing 0.0025-20 µg doses of mRNA-1273 in BALB/c mice was also evaluated to assess immunogenicity and demonstrated a positive dose-dependent production in pseudovirus-neutralizing antibodies.[1] Additionally, a prime-only regimen with 1 µg or 10 µg doses was evaluated and showed binding antibody titers without neutralizing antibody titers in both regimens.[1] Mice that received prime/boost regimen of 1 µg of mRNA-1273 were completely protected from mouse-adapted SARS-CoV-2 viral replication in the lungs when challenged 5 or 13 weeks after vaccination, and 6 of 7 mice did not have viral replication in the nasal turbinates.[1] Mice that received 0.1 µg prime/boost doses of mRNA-1273 had a reduction of 100-fold in viral load in the lungs, and those that received 0.01 µg prime/boost doses had a 3-fold lung viral load reduction.[1] When re-challenged 7 weeks after a prime-only regimen of 1 µg or 10 µg dose of mRNA-1273, there was no evidence of viral replication in the lungs of immunized mice.[1] The results of the preclinical trials of mRNA-1273 demonstrated that a prime/boost regimen of 1 µg of mRNA-1273 induced pseudovirus-neutralizing antibodies and CD8^{+} T cell responses, as well as more than 3 months protection from viral replication in the respiratory tract.[1]

The preclinical studies of mRNA-1273 also evaluated for the theoretical possibility of vaccine associated enhanced disease, which has previously been described with SARS-CoV and MERS-CoV and in some cases of RSV, measles, dengue and Zika.[1,54] Subprotective doses of 0.1 μg or 0.01 μg of mRNA-1273 did not demonstrate any evidence of increased mucous production or enhanced lung pathology in mice.[1]

V. Clinical data of mRNA therapeutics for SARS CoV-2:

Given the encouraging results from the preclinical studies of the mRNA-1273, BNT162b1 and BNT162b2 vaccine candidates, these were moved forward into clinical trials to further evaluate dosing regimen, safety, immunogenicity and efficacy.

Pfizer-BioNTech:

In a Phase I/II observer-blind, placebo-controlled study of the BNT162b1 vaccine candidate, 45 healthy adults aged 18-55 years old received 2 vaccines at 10 μg or 30 μg separated by 21 days, 100 μg prime only, or placebo.[53] RBD-binding IgG titers and SARS-CoV-2 neutralizing antibodies were measured at baseline and days 7, 21, 28 and 35.[53] The RBD-binding IgG were seen to persist at high levels after the last day of follow up (day 35) in the 10 μg and 30 μg prime/boost regimen subjects, with levels being 8 and 46.3-times the GMC seen in convalescent sera, and neutralizing GMT at 1.8 and 2.8-times convalescent sera.[53] Subjects who received a single dose of 100 μg did not have increased levels of RBD-binding IgG past day 21, but did demonstrate 3-times the GMC and 0.35-times the GMT of convalescent sera.[53] Local and systemic reactions were mainly mild to moderate in severity, transient and dose-dependent after 10 μg and 30 μg prime-boost regimens.[53] Fever was more common after the second dose of 30 μg compared with the 10 μg regimen (75% vs 10%), and no severe adverse events were reported.[53] There was no increased immunogenicity after prime-only of 100 μg but there was an increase in systemic and local reactions.[53]

In the phase I/II open-label non-randomized trial in Germany to evaluate immunogenicity and safety of the prime-boost regimen of BNT162b2, healthy participants age 19-55 were given prime-boost doses in 1 μg, 10 μg, 20 μg or 30 μg dose levels.[55] Immunogenicity assessments included S1-binding and RBD-binding IgG assays and SARS-CoV-2 neutralizing assays, which were

measured at baseline and on days 7, 21, 29, 43, 50 and 85 for all dose levels except 1 μg.[55] At all doses, a robust immune response, both humoral and T-cell mediated, was seen with no serious adverse events reported.[55]

In order to select the final vaccine candidate, an observer-blind, placebo-controlled dose escalation phase I study comparing prime/boost regimens of the two front-runner Pfizer-BioNTech vaccine candidates, BNT162b2 and BNT162b1, at 10 μg, 20 μg, and 30 μg dose levels was conducted to evaluate safety and immunogenicity.[56] One hundred and ninety-five adults 18-55 and 65-85 years old were randomly assigned to receive one of the two vaccine candidates or placebo.[56] Each participant received 2 doses of vaccine 21 days apart or placebo according to their allocation.[56] Assessments of immunogenicity were conducted at baseline and on days 7, 21, and 35 and included a SARS-CoV-2 serum neutralization assay and RBD-binding or S1-binding IgG assays.[56] Local reactogenicity was reported frequently after BNT162b1 vaccination, primarily injection site pain within 7 days of injection, in a dose dependent manner and the large majority were mild-moderate in severity.[56] Similar results were seen after BNT162b2 vaccination, with no grade 4 local reactions reported in either group.[56] Systemic side effects were milder in the BNT162b2 group, with 17% of the 18-55 age group reporting fever after the second dose and 8% in the 65-85 age group, while in the BNT162b1 group 75% of the 18-55 age group and 33% of the 65-85 group reported fever after the second dose.[56] Overall, BNT162b2 demonstrated a milder systemic reactogenicty profile compared with BNT162b1, especially in the older adults.[56] Although both demonstrated equivalent immunogenicity, BNT162b2 at 30 μg was selected over BNT162b1 to continue into phase II/III trials due to better tolerability.[50] The reasons for the difference seen in reactogenicity between the two constructs is unclear, however the BNT162b1 molecule is about 5 times larger in size than BNT162b2 and perhaps this was a contributing factor given the direct immunostimulatory properties of RNA.[56]

The safety and efficacy of BNT162b2 was evaluated in a phase II/III observer-blinded, placebo-controlled, multinational clinical trial of 43,548 participants 16 years or older who were randomized in a 1:1 allocation to receive 30 μg doses of prime/boost vaccine separated by 21 days.[57] The primary end point is the vaccine efficacy against laboratory confirmed COVID-19 and the study continues to monitor for safety.[57] At the first interim analysis, after 2 months of median follow up, 8 cases of PCR confirmed COVID-19 were seen in the BNT162b2 recipients (7 days after second dose), whereas 162 cases of PCR confirmed COVID-19 were seen in the placebo group.[57] This resulted in a vaccine efficacy of 95% (95% CI, 90.3 to 97.6).[57] These results were consistent across age, race/ethnicity, sex, body mass index (BMI) and pre-existing medical comorbidities.[57] Most local and systemic reactions were mild to moderate in severity, less common in those above age 55, and included transient pain at the injection site, fatigue, and headache.[57] Serious adverse events occurred in 0.6% of the BNT162b2 recipients and 0.5% of placebo recipients and included a ventricular arrhythmia, shoulder injury due to receipt of the vaccine, axillary lymphadenopathy, and leg paresthesia.[57] There were no deaths attributed to the vaccine.[57] Based on this data, the US

Food and Drug Administration (FDA) granted the BNT162b2 vaccine emergency use authorization (EUA) on December 11, 2020 and the study was transitioned to an open-label period. Post-EUA with the subsequent vaccination of millions of individuals, a few rare vaccine-related adverse effects have been described. Severe acute allergic reactions (anaphylaxis) were reported by the Centers for Disease Control (CDC) to have occurred in approximately 11.1 cases per million doses after 21 cases were reported to the Vaccine Adverse Event Reporting System (VAERS) from December 14 to 23, 2020, which occurred at a median interval of 13 minutes after receipt of vaccine (range 2-150 minutes).[58] No deaths due to anaphylaxis were reported and the CDC issued guidance of vaccine-related anaphylaxis encouraging prompt identification and management.[58] The secondary efficacy analysis was conducted with up to 6 months of post-vaccination data and continued to demonstrate high efficacy against COVID-19 (91% efficacy, 95% CI 89.0-92.3).[59] It demonstrated a VE of 96.7% against severe disease (95% CI 80.3-99.9) and in data from South Africa, where the Beta variant was dominant, it demonstrated 100% VE.[59] No new safety signals were identified and the study participants will continue to be followed for two years.[59] On August 23, 2021 the FDA accepted Pfizer-BioNTech's Biologics License Application and granted full approval of the BNT162b2 for people 16 years and older for the prevention of COVID-19 disease.

On September 22, 2021, the FDA amended the EUA to allow for a single 30μg booster dose at least 6 months following the completed primary vaccine series for people ≥ 65, people 18-64 years at high risk for COVID-19 or with increased institutional or occupational exposure to SARS-CoV-2. In the results from the phase I/II/III trial, VE up to 6 months post-dose 2 waned slightly over time (initial VE 96.2% from 7 days to <2 months post-dose 2 to 90.1% from >2 months to <4 months post-dose 2, and was 83.7% at >4 months post-dose 2).[60] In light of these data, Pfizer-BioNTech administered a 30μg booster dose a small group of participants during phase 1 of the ongoing clinical trial (11 participants 18-55 years of age and 12 participated 65-85 years of age).[61] VE 95.6% against COVID-19 was seen from results of the Phase 3 randomized controlled trial assessing the safety and efficacy of a 30μg booster dose with a median of 11 months between the booster and completion of the primary vaccine series.[61]

More data are emerging regarding the potential of the mRNA vaccines to decrease asymptomatic COVID-19 and transmission of COVID-19. A real-world observational study of recipients of BNT162b2 vaccine demonstrated a 2.8-4.5 fold reduction in viral load in infections 12 to 37 days after the first dose of vaccine.[62] Whether or not this correlates with severity of disease or transmissibility is yet to be determined.

As of May 10, 2021, the US FDA granted EUA authorization of BNT162b2 for adolescents ages 12-15 based on interim results from the ongoing randomized, placebo-controlled, observer blinded US clinical trial of 2,260 participants.[63] Local and systemic side effects were common and

dose dependent, largely mild to moderate in severity and transient including fatigue, myalgias, arthralgias, fever, and chills.[63] Immunogenicty in this age group was evaluated and compared with that demonstrated by the adult cohort ages 16-25 years, and was found to be non-inferior (geometric mean ratio 1.76 of SARS-CoV-2 50% neutralizing titers 1 month post-dose 2 in 12-15 year olds (GMT 1283.0) relative to 16-25 year olds (GMT 730.8)).[63] Among 1,005 vaccine recipients there were no PCR confirmed cases of COVID-19, whereas there were 16 cases of confirmed COVID-19 in 978 placebo recipients, with a vaccine efficacy of 100%.[63]

Data on children 5-11 years of age has demonstrated a VE 90.7% in preventing COVID-19 in this age group without any serious side effects noted in about 3,100 children in an ongoing study. On October 29, 2021 the US FDA granted EUA for use in children 5-11 years of age. Pfizer-BioNTech is currently conducting a phase 1/2/3 clinical trial evaluating a 2 dose-schedule of BNT162b2 in 3 dosing regimens in children 6 months to 11 years old.

Moderna

In a phase 1 open-label trial involving 45 healthy adults ages 18-55, the mRNA-1273 vaccine candidate was evaluated in a dose-escalation study of a prime/boost regimen of 25 μg, 100 μg, or 250 μg doses separated by 28 days to evaluate safety, immunogenicity, and reactogenicity. Systemic adverse events were largely mild to moderate with greater reactogenicity, in both severity and frequency, demonstrated in a dose dependent fashion where 21% reported one or more severe adverse events after the 250 μg doses.[64] It demonstrated potent immunogenicity with neutralizing antibodies to S-2P and the RBD in a dose dependent manner after just one dose in all participants that was comparable to levels seen in convalescent serum.[64] Of the three doses evaluated, the 100 μg prime/boost regimen was associated with robust immunogenicity and an improved reactogenicity profile compared with the 250 μg dose regimen (S-2P ELISA GMT at day 57 was 782,719 in the 100 μg-group and 1,192,154 in the 250 ug-group; for comparison, convalescent serum GMT is 142,140).[64]

The phase 2a placebo-controlled, observer-blinded, dose-confirmation trial in 600 healthy adults ages 18-55 and a sentinel group of 55 and older, to evaluate reactogenicity, safety, and immunogenicity of prime/boost regimen of 50 μg, 100 μg, or placebo 28 days apart.[65] The data on this is still preliminary, however both binding and neutralizing antibodies were seen after both dosing regimens in a dose dependent manner after the first dose but more equivalent after the second dose.[65]

The phase 3 placebo-controlled, observer-blinded study evaluated the prime/boost regimen of 100 μg versus placebo, separated by 28 days, in 30,420 SARS-CoV-2-naïve participants to evaluate for prevention of COVID-19 illness with onset 14 days after second vaccination.[66] At the interim analysis, with a median follow up of 64 days, there were 11 cases of symptomatic COVID-19 in the mRNA-1273 vaccine recipient group and 189 cases in the placebo group, with a reported vaccine efficacy of 94.1% (95% CI, 89.3 to 96.8%; P<0.001).[66] Reactogenicity was moderate, transient, and more common in the vaccine group.[66] There were 30 cases of severe COVID-19 and all were in the placebo group.[66] Severe adverse events were rare with a similar incidence in both groups.[66] The US FDA granted the mRNA-1273 vaccine EUA on December 18, 2020 and the study design was transitioned to open-label. The secondary efficacy analysis occurred at the completion of the blinded phase of the study, with a median follow up of 148 days, and continued to demonstrate high efficacy at preventing COVID-19 illness (93.2% efficacy, 95% CI 91.0 to 94.8%). It also continued to demonstrate high efficacy at preventing severe COVID-19 with only 2 cases of severe COVID-19 in the vaccine group and 106 in the placebo group, a VE of 98.2% (95% CI 92.8 to 99.6%).[67] Asymptomatic SARS-CoV-2 infections were also reduced (VE 63%).[67] The mRNA-1273 vaccine continued to show an acceptable safety profile and study participants will be followed for two years. The safety and efficacy reported in these clinical trials to date is summarized in Table 2 and Table 3 respectively.

In a preprint of an exploratory analysis of the Moderna study, the incidence of COVID-19 infection during delta variant predominate period (July to August 2021) were compared in those who were vaccinated early (14,746 participants vaccinated from July to December 2020, 13 months median follow up, mRNA-1273e) versus later (11,431 participants vaccinated from December 2020 to April 2021, 7.9 months of median follow up, mRNA-1273p).[68(p19)] During this period of time, 162 COVID-19 cases were seen in the mRNA-1273e group and 88 COVID-19 cases in the mRNA-1273p group (incidence rate 49.0 per 1000 person years in the later vaccinated group compared with 77.1 per 1000 person years in the early vaccinated group).[68(p19)] Additionally, the severe COVID-19 cases were fewer in the later vaccinated mRNA-1273p compared with the mRNA-1273e group. Most of the sequenced cases were attributed to the delta variant (97% in mRNA-1274e and 99% in mRNA-1273p groups).[68(p19)]

On October 20, 2021 the FDA amended the EUA to allow for a 50μg dose of mRNA-1273 as a booster dose at least 6 months after completion of the primary vaccine series for people ≥ 65, people 18-64 years at high risk for COVID-19 or with increased institutional or occupational exposure to SARS-CoV-2. This was based on review of data from the Phase 2 clinical trial, during which a 50μg dose of mRNA-1273 as a booster dose was given to 344 interested participants 6-8 months following the second dose of a priming series. Per Moderna press release on October 20, 2021, waning of neutralizing antibody titers was seen at 6 months, particularly against the VOC, but a booster dose of 50μg dose of mRNA-1273 significantly elevated neutralizing titers. These effects

were seen across all age groups and the reported safety profile was similar to the post-dose 2 profile.[69]

Studies in adolescents have also reported promising results and are currently underway. The phase 2/3 TeenCove study includes 3,732 adolescents from 12 to less than 18 years of age who were randomized 2:1 to receive prime/boost of 100 μg of mRNA-1273 or placebo.[70] Based on their interim analysis, vaccine efficacy was reported at 100% with 4 cases of COVID-19 in the placebo group and none in the vaccinated group, and no severe adverse events reported.[70] The analysis of the immunogenicity subset (which included 289 adolescents and 289 young adults (18-≤25)) demonstrated the immune response in adoescents was non-inferior to young adults (geometric mean ratio 1.08 of SARS-CoV-2 50% neutralizing titers 1 month post-dose 2 in 12-<18 year olds (GMT 807) relative to 18-≤25 year olds (GMT 740)).[70] Side effects in this adolescent group were comparable to the adult group with the large majority reported as mild to moderate in severity.[70]

Evaluations in children have been initiated. Moderna is currently conducting a phase 2/3 two-part dose escalation, open label, observer blinded, placebo controlled clinical trial of a 2-dose regimen of mRNA-1273 in children 6 months to 11 years old.[71]

Special Populations:

Immunocompromised

Immunocompromised patients, including those on immunosuppressive medications, and those with autoimmune conditions were specifically excluded from the clinical trials. No safety concerns have been identified in this group but current data shows that the mRNA vaccines are less effective at stimulating an immune response in this population.[72] A large study of 658 transplant recipients found that only 15% were able to produce measurable antibodies after dose 1 and 2 and 46% did not have an antibody response.[72] A double-blind, placebo-controlled, randomized study of 120 solid organ transplant recipients demonstrated that a third dose of mRNA-1273 provided increased median percent virus neutralization (71% mRNA-1273 group vs 13% placebo) and improved neutralizing antibodies (60% mRNA-1273 group vs 25% placebo achieved above the 30% threshold).[73] In a French study evaluating 101 solid-organ transplant recipients who previously received 2 doses of BNT162b2 vaccine, 44% seroconverted after a third dose of BNT162b2 (26 of 59 patients).[74]Data continue to emerge emphasizing that solid organ transplant recipients remain a particularly vulnerable population despite receipt of a 2-dose primary vaccination series.[75,76] Reduced antibody responses after a two-dose primary vaccination series has also been observed in those with chronic lymphocytic leukemia (CLL) after vaccination with BNT162b2, with a antibody response rate of 39.5% reported in one study.[77] The study noted that those in clinical remission were most likely to respond to vaccination (79.2%) whereas those who hadn't yet received treatment for their malignancy responded at a lower rate (55.2%).[77] The

responses in those actively receiving treatment were poor (16%) and none of those who received an anti-CD20 agent within 12 months of vaccination responded.[77] Not much is known yet about the magnitude of the role of cell-mediated immune responses to COVID-19 in this population, however recent data suggests that CD4+ T-cell responses to the S-protein may positively correlate with anti-SARS-CoV-2 IgG and IgA titers.[78] Data from the VISION Network compared VE against COVID-19 associated hospitalization after receipt of a 2-dose vaccination series in 20,101 immuncompromised adults and 69,116 immunocompetent adults.[79] VE was found to be lower in the immunocompromised patients (77%) compared with the immunocompetent patients (90%) irrespective of age, delta variant predominance, and mRNA vaccine product.[79] VE point estimates demonstrated a VE of 79% in those with solid malignancy, 74% with hematologic malignancy, 81% with a rheumatologic or inflammatory disorder, 73% with an intrinsic immune or immunodeficiency, 59% in solid organ or stem cell transplant recipients. Generally, the VE point estimates were higher for those who received 2 doses of Moderna compared with Pfizer-BioNTech except in those with a rheumatologic or inflammatory disorder (they were the same).[79] On August 12, 2021, the FDA amended the EUA for the Pfizer-BioNTech and Moderna COVID-19 vaccines, which allowed moderately to severely imunocompromised individuals to receive an additional dose of COVID-19 vaccination for a 3 total dose primary vaccine series. One day later the CDC issued a statement supporting the FDA's amendment. Several studies have demonstrated that 33-50% of immunocompromised people developed detectible antibodies after a 3rd dose when they had not after a 2-dose primary vaccination series.[74,80–82] The CDC has since allowed for this population to receive a 4th dose of mRNA COVID-19 vaccination. Additional data regarding the VE after a 3-dose primary vaccination series are needed.

Pregnancy

Preliminary findings evaluating safety of mRNA-1273 and BNT162b2 in 35,691 pregnant women from ages 16 to 54 via the V-safe surveillance data system did not show any concerning safety signals in pregnancy or neonatal outcomes.[83] A descriptive, exploratory study in a cohort of 103 women including 30 pregnant and 16 lactating women, evaluated the immunogenicity after mRNA COVID-19 vaccination.[84] There was demonstrated immunogenicity in this population of women, including humoral and T cell mediated immunity, and vaccine associated antibodies were conferred via cord blood and breast milk to the infant.[84] Both binding and neutralizing antibodies were also seen against two variants, B.1.1.7 (Alpha) and B.1.351 (Beta).[84] Data from Norway found no evidence to suggest early pregnancy loss after COVID-19 vaccination, with a reported adjusted odds ratio of 0.8 (95% CI 0.67-0.96) after Pfizer-BioNTech and 0.84 (95% CI 0.56-1.25) after Moderna within the prior 5 weeks of vaccination.[85] Another study including 105,466 pregnancies showed that 8.0% of women with ongoing pregnancies had received a mRNA COVID-19 vaccine in the prior 28 days relative to an index date vs 8.6% of spontaneous abortions and the spontaneous

abortions were not associated with an increased odds of COVID-19 vaccine exposure compared with ongoing pregnancies (adjusted odds ratio 1.02; 95% CI 0.96-1.08).[86] Emerging data continues to show that COVID-19 vaccination (including BNT162b2 and mRNA-1273) in pregnant women is safe, effective, and immunogenic, allowing transfer of antibodies to the neonates.[87–89] In August 2021 the CDC recommended COVID-19 vaccination for pregnant women.

Adolescent and Pediatric populations are discussed in their respective sections above.

Side effects:

Although the phase 3 studies of both mRNA-1273 and BNT162b2 included rigorous evaluations for safety and side effects, the post-authorization administration of mRNA vaccines to millions of people has identified rare events that were not seen in the clinical trials as they were not powered to detect these. In the most rigorous vaccine safety monitoring effort in US history, several vaccine safety-monitoring systems have been implemented to further identify and track adverse events during the mRNA vaccine rollout. The Vaccine Safety Datalink (VSD) includes 9 healthcare sites in collaboration with the CDC and links patient vaccination and EMR data. VAERS collects reports of AEs post-vaccination nationwide. V-Safe lets users use their smartphone to report post-vaccination symptoms and health concerns, it also has a pregnancy registry to monitor those who received a vaccine just before or during pregnancy. The Clinical Immunization Safety Assessment Project (CISA) is a panel of vaccine safety experts from the CDC who collaborate with 7 research centers across the US to monitor vaccine safety.[90] VAERS and V-Safe are self-reporting modalities that are available to the public and allow for rapid identification of potential safety signals. The limitation is that this information is subject to reporting bias and potentially incomplete data.

Rare cases of anaphylaxis after COVID-19 mRNA vaccination were reported via VAERS with a reporting rate of 4.7 cases/million doses administered after Pfizer-BioNTech vaccine and 2.5 cases/million doses administered after Moderna vaccine.[91] A larger study of 64,900 hospital employees reported severe allergic reactions consistent with anaphylaxis at a rate of 2.47 per 10,000 mRNA COVID-19 vaccination.[92] Although the mechanism of anaphylaxis is still unclear, 31% of those with anaphylactic reactions had a history of anaphylaxis.[92] Rare cases of myocarditis and pericarditis have been seen post-mRNA COVID-19 vaccination.[93,94] Although the incidence is difficult to estimate with data rapidly accumulating, VAERS data up 10/6/21 showed 3,336 cases of

myocarditis and pericarditis (2,459 myopericarditis, 877 pericarditis alone), primarily in younger males within 7 days after the second dose of mRNA vaccine (reporting rates of myocarditis per 1 million doses administered within 7 days after the second dose of an mRNA vaccine ranged from 2.8-37.4 in those 12-39 years of age).[95] From a retrospective review of data from Israel, the incidence of myocarditis remained rare but was increased after vaccination, particularly in males 16-19 years of age (rate ratio 30 days after second vaccination 8.96 in comparison to unvaccinated persons).[96] Another large database study from Israel estimated the incidence of myocarditis after at least 1 dose of mRNA vaccine was 2.13 cases per 100,000 people, with the highest in males 16-29 years of age (10.69 cases per 100,000 persons).[97] Data from the Vaccine Safety Datalink (VSD) demonstrated an adjusted rate ratio of 1.72 for myocarditis/pericarditis in the 21 day interval after any dose of an mRNA vaccine.[98] A subgroup analysis further demonstrated in data through 10/9/21 that those age 12-39 are at increased risk, especially through 7 days post vaccination, with significantly higher rates after Moderna than after Pfizer-BioNTech (8.0 excess cases in the risk period per 1 million doses of Moderna versus Pfizer-BioNTech within 2 days after either vaccine, 13.3 within 7 days of the second dose).[98] Other studies which show a greater risk of myocarditis/pericarditis after Moderna than Pfizer-BioNTech include studies from Canada[99] and Scandinavia[100]. However, other US vaccine safety monitoring systems including VAERS, FDA BEST Systems and the VA, did not show a difference in rate between the two mRNA vaccines. The incidence of myocarditis after dose 3 of mRNA vaccines is not yet available as data is only available in a small number of volunteers from the clinical trials, but is forthcoming.

Effectiveness of mRNA vaccines

The clinical trials continue to demonstrate the efficacy and safety of the COVID-19 vaccines and emerging real-world data corroborates these findings. Table 4 summarizes the realworld effectiveness data from selected key studies. Data from Israel after launching its COVID-19 vaccination program on December 20, 2020, showed that vaccine efficacy of the BNT162b2 vaccine was 92% for any SARS-CoV-2 infection, 94% for symptomatic COVID-19, 87% for COVID-19 related hospitalization, and 92% for severe COVID-19.[101] Real-world data of vaccine effectiveness in 7,280 older adults ≥65 years from the COVID-19-Associated Hospitalization Surveillance Network (COVID-NET) re-demonstrated high effectiveness in this population.[102] Of fully vaccinated adults aged 65-74 years, vaccine efficacy at preventing COVID-19-associated hospitalization was 96% for both BNT162b2 and mRNA-1273 recipients.[102] In those who were fully vaccinated but ≥ 75 years, the efficacy was 91% for BNT162b2 and 96% for mRNA-1273 recipients.[102]

Ongoing assessments of vaccine effectiveness remains important as SARS-CoV-2 variants of concern (VOC) continue to emerge in the setting of concern for waning vaccine-induced immunity.[103] The data on the durability of vaccine-induced immunity and VE against the current VOC, including the Alpha, Beta, and Delta variants, is variable. This is likely due to differences in study population and design including variation in methods of data analysis, end points of interest, diagnostic testing utilized, and prevalence of SARS-CoV-2 variants.[103,104] There has been suggestion of reduced neutralization *in vitro* against the Beta variant in individuals vaccinated with BNT162b2.[105] A case-controlled observational study in Quatar, which assessed the vaccine effectiveness of BNT162b2 against any infection with the Beta or Alpha variants, estimated vaccine effectiveness against the Beta variant was 75% and 89.5% against the Alpha variant.[106] However, demonstrated effectiveness against severe, critical, or fatal disease with COVID-19 remained high at 97.4%.[106] Several additional studies have continued to demonstrate that despite the aparent *in vitro* reduction of neutralization against the Beta variant, vaccine effectiveness does not appear to diminish.[107] In an observational study evaluating the effectiveness of the mRNA vaccines against infection with SARS-CoV-2 in residents of nursing homes, vaccine effectiveness demonstrated a notable decline, from 74.7% (74.2% for Pfizer-BioNTech, 74.7 for Moderna) to 53.1% (52.4% for Pfizer-BioNTech, 50.6% for Moderna), in the pre-Delta period compared with the Delta-predominant period in the US.[103] Data from the UK found vaccine effectiveness against symptomatic COVID-19 after BNT162b2 to be 88.0% against the Delta variant (compared with 93.7% against Alpha).[108] Retrospective data from the US demonstrated a VE of 73% (95% CI 72-74) against SARS-CoV-2 infection after receipt of BNT162b2 vaccine series, but did show a decline from 1 month to 5 months after vaccination (88% and 47% VE respectively).[109] Another study conducted across 5 US VA hospitals showed a VE against COVID-19 related hopsitalizations of 87% even during a period of widespread delta variant prevalence. (Pfizer-BioNTech 83.4%; 95% CI 74-89.4; Moderna 91.6%; 95% CI 83.5-95.7)[110] Data from the HEROS-RECOVER Cohort showed a VE of 80% (95% CI 69-88) against SARS-CoV-2 infection during the study period of December 14, 2020 to August 14, 2021.[111] Additional studies using realworld data continue to support these findings (Table ***).[103,112–115] Both the BNT162b2 and mRNA-1273 continue to demonstrate evidence of high and sustained protection against severe COVID-19 disease, even in high risk populations, through 24 weeks after vaccination.[116] Additionally, studies continue to show a reduction in severe COVID-19-associated illness in highly vaccinated populations.[117] One study reported that cumulative hospitalizations were 17 times higher in the unvaccinated from January 24, 2021 to July 24, 2021 compared with vaccinated people.[118] Moderna has developed two additional vaccine candidates, mRNA-1273.351 targeting the Beta variant and mRNA-1273.211 with a 50/50 mix of mRNA-1273 and mRNA-1273.351, and has entered into clinical trials with these constructs.[119] Preclinical studies from mice show that mRNA-1273.351 successfully increased neutralizing antibodies to the Beta variant, while the mRNA-1273.211 induced broad neutralization against multiple VOC (D614G, B.1.351, P.1, B.1.427/B.1.429).[119] Whether future third dose booster vaccinations will target VOC or the initial reference strain is an area of ongoing evaluation. In a multistate study of 3,089 hospitalized adults, VE against COVID-19-associated hospitalization was found to be sustained with 86% at 2 to 12 weeks post vaccination and 84% at 13 to 24 weeks post vaccination for patients fully vaccinated against SARS-CoV-2 with a COVID-19 mRNA vaccine (mRNA-1273 or BNT162b2).[116] These data

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were consistent even across subgroups at high risk for severe disease, although adults with an immunocompromising condition had an overall lower vaccine efficacy.[116] The secondary analysis data of Pfizer-BioNTech's BNT162b2 vaccine demonstrated a peak VE against lab-confirmed COVID-19 of 96.2% from 7 days to less than 2 months after the second vaccine dose, however went on to demonstrate that VE declined an average of 6% every 2 months thereafter.[59] Some emerging data have suggested that the VE for Moderna is higher than for Pfizer-BioNTech (93% vs 88%) based on realworld data among US adults.[120]

Realworld VE data of Pfizer-BioNTech against COVID-19 associated hospitalization in adolescents has so far demonstrated a VE of 93% among 464 hospitalized persons aged 12-18 .[121]

Heterologous Boost

Given the available data on homologous booster vaccinations has been shown to be safe and immunogenic, evaluations of heterologous booster vaccinations are underway. In a preprint of a US phase 1/2 non-randomized, open-label clinical trial, previously vaccinated adults received one of three vaccines (Moderna 100ug, Jannsesn Ad26.COV2.S $5x10^{10}$ virus particles, or Pfizer-BioNTech BNT 162b2 30ug).[122] The initial data demonstrated that heterologous booster vaccines resulted in similar or higher neutralizing antibody titers than homologous booster vaccines (6.2-76 fold vs 4.6-56 fold), with mRNA vaccines demonstrating the higher antibody titers within 28 days of the boost.[122]

CureVac:

The mRNA vaccine candidate from CureVac (CVnCoV) encoding the stabilized full-length spike protein encapsulated in a LNP was initially a promising contender of very similar design to the mRNA-1273 and BNT162b2.[123] In the phase 1 assessment, all investigated doses provoked an immune response with 56-77% demonstrating VNT seroconversion 2 weeks after lower doses (2-8 ug dose level) and 100% after the 12 μg dose level.[124] In a phase 2b/3 international randomized, double-blinded, placebo-controlled study of 40,000 adults 18 years of age or older, the interim vaccine efficacy was 47% against COVID-19 disease of any severity.[123] Given these disappointing efficacy results, to the vaccine will not move forward. The reasons for the failure of CVnCoV despite being very similar to the mRNA vaccine constructs from Pfizer-BioNTech and Moderna are unclear but may be related to the use of modified mRNA. The CVnCoV vaccine contained uridine instead of the modified nucleoside pseudouridine found in the Pfizer-BioNTech and Moderna products.[107,123] This may have resulted in differences in mRNA translation or innate immunity responses.[107] Several

other mRNA vaccine constructs are currently in phase I to III clinical trials but at this time the majority lack clinical data and therefore will not be discussed in this review.

VI. Discussion:

New and emerging pathogens continue to threaten human health globally and preparedness and pandemic response are key for early containment of outbreaks. Coronaviruses are zoonotic diseases that have previously been predicted to be highly capable of causing pandemics.[1] In the last 20 years SARS-CoV-2 is the third novel betacoronavirus to cause significant disease after SARS-CoV, first reported in China in 2002, and MERS-CoV, first reported in Saudi Arabia in 2012.[1]

Since the first studies exploring mRNA therapeutics in the 1970s, the collective scientific effort to evaluate and develop mRNA as a viable preventative therapeutic for infectious diseases has made tremendous progress and demonstrated promising results. Despite the common misconception that mRNA vaccines are a new development, they are actually the culmination of decades of scientific research and have been under research for years for various infectious diseases including Ebola, CMV and Zika viruses. The mRNA vaccines BNT162b2 and mRNA-1273 have played a key role in our global response to the COVID-19 pandemic as they have demonstrated significant advantages over conventional vaccines. Compared with the other available vaccines against SARS-CoV-2, the mRNA vaccines have demonstrated higher antibody responses.[107] While many of the studies to date utilize convalescent serum as a comparator, the correlate of protection is still unknown and this is an area of ongoing investigation although there is mounting evidence that neutralizing antibodies to the spike protein play an important role.[125,126] Data from the UK evaluating humoral immune responses to with the ChAdOx1 nCoV-19 vaccine suggests that an 80% VE against symptomatic SARS-CoV-2 (in the Alpha variant dominated setting) was demonstrated with 264 anti-spike binding antibody units (BAU/mL), 506 BAU/mL for anti-RBD antibodies, 26 IU/mL pseudovirus neutralization, and 247 normalized live-virus neutralization titers.[127] It is possible that these responses are dependent on different vaccine platforms, therefore conclusions should be put into the appropriate context until more data is available. Data from the mRNA vaccines is still in the preprint form, however appears to show a similar trend.[128] While the role of T-cell responses in protective immunity against SARS-CoV-2 is still an ongoing area of research, some evidence suggests that CD4+ T-cell response to the S-protein were vigorous after vaccination and correlated with anti-SARS-CoV-2 IgG and IgA levels.[78] T-cells have been demonstrated to play a role in prevention of progression of disease and duration of RNA shedding, however their role as a correlate of protection is still unknown.[129] Goel et al. showed that SARS-CoV-2 specific memory B cells increased in the 3-6 months after mRNA vaccination, despite a decline in antibody levels.[130]

Several studies support the finding that both antigen specific CD4+ and CD8+ T cells are induced by mRNA vaccination against COVID-19 and appear to be durable through 6 months post-vaccination.[130–132] Given the observation that protection against severe COVID-19 and death persists despite evidence of waning antibodies, it is reasonable to hypothesize that cellular immunity including memory responses likely significantly contribute, however further studies are required. In line with this hypothesis, the afforded protection against severe Covid-19 has been challenged with two viral variants so far, Delta and Omicron, which were associated with a ~3 and 25-50 fold reduction in neutralization by antibodies raised against the vaccine strain. [133, 134] A large study from Kaiser Permanante has shown that the emergence of the delta variant resulted in diminution of protection against infection with the Delta variant but not against Covid-19 hospitalizations, which remained at 94.5%. 6-8 months after vaccination.[135]Early epidemiologic data from the US indicate that infections with the Omicron variant occur after previous infection, 2 doses of mRNA Covid-19 vaccines, and boosting albeit with characteristically mild outcomes.[136]

This is a rapidly evolving area of study and data continue to emerge. The COVID-19 mRNA vaccines from Pfizer-BioNTech and Moderna have proven to be highly efficacious against COVID-19-associated hospitalization and severe disease. Their efficacy has been demonstrated across different age groups, racial and ethnic groups, and coexisting medical comorbidities. Due to the success of the mRNA vaccines during the COVID-19 pandemic, Pfizer-BioNTech and Moderna are exploring their application to other infectious disease pathogens and COVID-19 may be just the beginning. The application of mRNA vaccines to a wide variety of other infectious disease pathogens is an area of rapid growth, including HIV, influenza, RSV, CMV, hepatitis C, Nipah virus, Zika virus, human metapneumovirus, and parainfluenza type 3. Some of these recent mRNA vaccine constructs are expected to enter phase 1 clinical trials as early as this year.

Acknowledgements:

Figure Legends

Figure 1. Mechanism of non-replicating mRNA vaccines against COVID-19

Figure 2. Timeline of Events in COVID-19 mRNA Vaccine Development

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Table 1. Preclinical Data Summary for mRNA COVID-19 Vaccines

Study Product	Animal Model	Dose (50% virus-neutralization GMT (μg))	Cell-mediated Immunity	Evidence of Protection
BNT162b2 Vogel et al[51]	Rhesus macaques	30 μg (962 in sera drawn 7 or 14 days post dose 2, 285 at 28 days post dose 2) 100 μg (1689in sera drawn 7 or 14 days post dose 2, 283 at 28 days post dose 2)	CD4+ T cells (TH1 predominant response) CD8+ T cells were elicited	No SARS-CoV-2 RNA on BAL samples 3 days after challenge (with intranasal and intratracheal 1.05 × 106 PFU of SARS-CoV-2 USA-WA1/2020 isolate)
BNT162b2 Vogel et al[51]	BALB/c mice	5 μg (296 28 days after 1 dose)	CD4+ and CD8+ T cell responses	-
BNT162b1 Vogel et al[51]	Rhesus macaques	30 μg (768 in sera drawn 7 or 14 days post dose 2) 100 μg (1714 in sera drawn 7 or 14 days post dose 2)	CD4+ T cells (TH1 predominant response) CD8+ T cells were elicited	SARS-CoV-2 RNA detected in BAL samples of 2/6 immunized macaques 3 days after challenge (with intranasal and intratracheal 1.05 × 106 PFU of SARS-CoV-2 USA-WA1/2020 isolate)
BNT162b1 Vogel et al[51]	BALB/c mice	5 μg (1,056 28 days after 1 dose)	CD4+ and CD8+ T cell responses	-

mRNA-1273[1]	BALB/c mice	1 µg (819 after 2 doses) 10 µg (315 between week 2 and 4 after 1 dose) 0.0025-20 µg doses trialed with a strong positive dose-dependent production of binding and pseudovirus-neutralizing antibodies	CD4^{+} and CD8^{+} T cell responses	Protected against viral replication in the lungs, protected and nares in 6 out of 7 mice

Table 2. Safety Analyses Data of mRNA COVID-19 Vaccines from Clinical Trials

Adverse Event	BNT162b2 Phase II/III clinical trial after 2 months of follow-up[57]	BNT162b2 Phase II/III clinical trial after 6 months of follow-up[59]	mRNA-1273 Phase III clinical trial after 2 months of follow-up[66]	mRNA-1273 Phase III clinical trial after 5.3 months of follow-up[67]
Any Local	-	-	84.2 (N=15168)	84.2 (N=15166)
Dose 1 (%)			88.6 (N=14677)	88.7 (N=14691)
Dose 2 (%)				
Any Systemic	-	-	54.9 (N=15168)	54.8 (N=15166)
Dose 1 (%)			79.4 (N=14677)	79.5 (N=14691)
Dose 2 (%)				
Any Serious (%)	0.6 (N=21621)	0.6 (N=21926)	0.6 (N=15185)	0.6 (N=15184)
Related to study vaccination (%)	26.7 (N=21621)	23.9 (N=21926)	4.5% (N=15185)	13.6% (N=15184)
Serious (%)	0 (N=21621)	0 (N=21926)	<0.1 (N=15185)	<0.1 (N=15184)
Fatal (%)	0 (N=21621)	0 (N=21926)	0 (N=15185)	0 (N=15184)
Medically attended (%)	-	-	0.9 (N=15185)	1.4 (N=15184)
Severe (%)	-	-	0.5 (N=15185)	0.5 (N=15184)

Table 3. Efficacy of the mRNA COVID-19 Vaccines from Clinical Trials

Trial	SARS-CoV-2 infection	COVID-19*	Severe COVID-19*	Death due to COVID-19
BNT162b2 Phase II/III clinical trial after 2 months of follow-up[57]	-	94.6 (95% CI 89.9-97.3)	75% (95% CI -152.6, 99.5)	100.0
mRNA-1273 Phase III clinical trial after 2 months of follow-up[66]	-	94.1 (95% CI 89.3-96.8)	100.0% (95% CI could not be estimated)	100.00
BNT162b2 Phase II/III clinical trial after 6 months of follow-up[59]	58.4% (95% CI 40.8-71.2)	91.3 (95% CI 89.0-92.3)	96.7% (95% CI 80.3-99.9) *as defined by the FDA	100.0
mRNA-1273 Phase III clinical trial after 5.3 months of follow-up[67]	63.0% (95% CI 56.6-68.5)	93.2% (95% CI 91.0-94.8)	98.2% (95% CI 92.8-99.6)	100.00

*see reference for definition of COVID-19 and severe COVID-19

Table 4. Real World Effectiveness of mRNA COVID-19 Vaccines from Selected Studies

Study	Vaccine	Study Period	Study Population	Vaccine Efficacy (%)
Tartof et al[106]	BNT162b2	12/14/20-8/8/21	≥12 years old Kaiser Permanente (California) patients	Symptomatic COVID-19 73 (95% CI 72-74) COVID-19 associated hospitalization 90 (95% CI 89-92)
Bajema et al[107] SUPERNOVA Network	BNT162b2, mRNA-1273	2/1/21-8/6/21	US veterans ≥ 18 years	COVID-19 associated hospitalization 87 (95% CI 90.4-91.1) BNT162b2 83.4 (95% CI 74-89.4) mRNA-1273 91.6 (95% CI 83.5-95.7)
Fowlkes et al[108] HEROS-RECOVER Cohort	BNT162b2, mRNA-1273, Ad26.COV2.S	12/14/20-8/14/21	Healthcare work	Any SARS-CoV-2 Infection 80 (95% CI 69-88)
Rosenberg et al[109]	BNT162b2, mRNA-1273, Ad26.COV2.S	5/3/21-7/19/21		Any SARS-CoV-2 Infection 91.8-75.0 COVID-19 associated hospitalization >89%
Thompson et al[110]	BNT162b2, mRNA-1273	1/1/21-6/22/21	Adults ≥50 y/o	COVID-19 associated hospitalization 89 (95% CI 87-91) 90 (95% CI 86-93) for ICU

				admission
				91 (95% CI 89-93) for ER/urgent care
Grannis et al[111] VISION Network	BNT162b2, mRNA-1273, Ad26.COV2.S	June-August 2021	Adults ≥18	COVID-19 associated hospitalization 86 (95% CI 82-89) BNT162b2 80 Moderna 95 82 for COVID-19 Ed/UC encounters

Study	Vaccine	Study Period	Study Population	Vaccine Efficacy (%)
Nanduri et al[100] NHSN	BNT162b2, mRNA-1273	March – August 2021	Nursing home residents	Any SARS-CoV-2 Infection 74.7 (95% CI 70.0-78.8) during pre-Delta (BNT162b2 74.2, mRNA-1273 74.7) 53.1 (95% CI 49.1-56.7) during Delta (BNT162b2 52.4, mRNA-1273 50.6)
Goldberg et al[112] Israeli national database	BNT162b2	7/11/21-7/31/21	≥16 years	Any SARS-CoV-2 Infection 52-83 Severe COVID-19 85-98

SUPERNOVA Network: Surveillance Platform for Enteric and Respiratory iNfectious Organisms at the VA; HEROS-RECOVER Cohort: Arizona Healthcare, Emergency Response and Other Essential Workers Surveillance Study (HEROES); Research on the Epidemiology of SARS-CoV-2 in Essential Response Personnel (RECOVER); VISION Network: VISION network includes Columbia University Irving Medical Center (New York), HealthPartners (Minnesota and Wisconsin), Intermountain Healthcare (Utah), Kaiser Permanente Northern California (California), Kaiser Permanente Northwest (Oregon and Washington), Regenstrief Institute (Indiana), and University of Colorado (Colorado); NHSN: National Healthcare Safety Network.